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16. ABSTRACT

The adult rat kidney is an important target organ for sodium fluoride; however, the toxicity of fluoride to the developing kidney is unknown. This study examined renal function following NaF exposure during the first four weeks after birth. Sprague-Dawley rats received a single ip injection of 0, 30, or 48 mg/kg NaF on postnatal day 1, 8, 15 or 29. Alterations in renal function, histology and morphology were determined 24, 38, and 120 hours after exposure. Measurements of renal function included urine volume, osmolality, the ability to concentrate urine during water deprivation, urinary pH and chloride content. Rats were then sacrificed and their kidneys processed for observation by light microscopy. Some minor alterations in renal function were observed in the three youngest age groups after NaF exposure. These changes included decreased body weight after treatment with 30 or 48 mg/kg NaF but increased kidney-body weight ratio in the 49 mg/kg group on day 1; decreased urinary pH in both dose groups after treatment on day 1 or day 8; increased uring volume 120 hours after treatment on day 9; and decreased chloride excretion in the 49 mg/kg group 24 hours after injection on day 1. None of these aberrations were severe. In contrast to these results, marked renal toxicity was observed in the post-weaning rats treated on day 29. The NaF exposure resulted in increased kidney weight and kidney/body weight ratio, profound diuresis, decreased urinary osmolality and decreased ability to concentrate urine during water deprivation. Urinary chloride excretion was decreased for the first two days after NaF exposure, then increased in water deprived rats 120 hours after treatment. Glucosuria and hematuria were present for two days after treatment with 49 mg/kg. Histological lesions were apparent in theproximal tubules of the treated day 29 rats. Thus, the kidney of the suckling rat is largely unresponsive to NaF toxicity. Renal sensitivity increases abruptly after weaning in the day 29 rat.

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Toxicity of Sodium Fluoride to the Postnatally Developing Rat Kidney

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The adult rat kidney is an important target organ for sodium fluoride; however, the toxicity of fluoride to the developing kidney is unknown. This study examined renal function following NaF exposure during the first 4 weeks after birth. Sprague–Dawley rats received a single ip injection of 0, 30, or 48 mg/kg NaF on postnatal Day 1, 8, 15, or 29. Alterations in renal function, histology, and morphology were determined 24, 48, and 120 hr after exposure. Measurements of renal function included urine volume, osmolality, the ability to concentrate urine during water deprivation, urinary pH, and chloride content. Rats were then sacrificed and their kidneys processed for observation by light microscopy. Some minor alterations in renal function were observed in the three youngest age groups after NaF exposure. These changes included decreased body weight after treatment with 30 or 48 mg/kg NaF but increased kidney–body weight ratio in the 48 mg/kg group on Day 1; decreased urinary pH in both dose groups after treatment on Day 1 or 8; increased urinary volume 120 hr after treatment on Day 8; and decreased chloride excretion in the 48 mg/kg group 24 hr after injection on Day 1. None of these effects was severe. In contrast to these results, marked renal toxicity was observed in postweaning rats treated on Day 29. The NaF exposure resulted in increased kidney weight and kidney/body weight ratio, profound diuresis, decreased urinary osmolality, and decreased ability to concentrate urine during water deprivation. Urinary chloride excretion was decreased for the first 2 days after NaF exposure, then increased in water-deprived rats 120 hr after treatment. Glucosuria and hematuria were present for 2 days after treatment with 48 mg/kg. Histological lesions were apparent in the proximal tubules of the treated Day 29 rats. Thus, the kidney of the suckling rat is largely unresponsive to NaF toxicity. Renal sensitivity increases abruptly after weaning in the Day 29 rat. © 1985 Academic Press, Inc.

INTRODUCTION

Fluoride is a ubiquitous element in the United States, largely because of extensive fluoridation of public water supplies as a preventive measure against dental caries. The fluoride ion is mainly distributed to bone where it is sequestered, and to the kidney where it is excreted (Largent, 1952; Chen *et al.*, 1956; Carlson *et al.*, 1960; Hall *et al.*, 1977). Fluoride causes toxicity in the kidney, where it produces histological lesions of the proximal tubule (Kosek *et al.*, 1972; Mazze *et al.*, 1973), a profound diuresis and diminished renal functional capabil-

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ities (Mazze, 1976). These functional effects may be the result of reabsorption deficits in the proximal tubule (Kosek *et al.*, 1972; Mazze *et al.*, 1973), inhibition of chloride transport in the ascending limb of the loop of Henle (Rush and Willis, 1982), or loss of responsiveness to vasopressin in the collecting tubule (Mazze, 1976; Rush and Willis, 1982).

Although the toxicity of NaF to the adult kidney has been well documented, the renal response of the postnatally maturing animal is unknown. We recently reported that there is an age-related increase in the renal toxicity of $HgCl_2$ in the rat as it matured from birth to 4 weeks of age (Daston *et al.*, 1983, 1984). It was of interest to us to determine whether this was specific to $HgCl_2$, or whether the resistance of the immature nephron extended to other renal toxicants with different mechanisms of action. $HgCl_2$ affects the proximal tubule, whereas NaF probably produces proximal tubular and medullary dysfunction. There is also some evidence that the toxicity of NaF is age related. The lethality of NaF has been reported to increase in the rat from 1 month of age onward (Maynard *et al.*, 1951; deLopez *et al.*, 1976); however, none of the toxicity studies was carried out in rats from birth to weaning. The present study was undertaken to determine the postnatal developmental response to NaF, a renal toxicant which affects a greater portion of the nephron than does $HgCl_2$, and which acts through a different mechanism.

MATERIALS AND METHODS

Animals. Time-pregnant Sprague–Dawley CD rats (Charles River Breeding Laboratories) were received in our laboratory on Day 15 of gestation (sperm positive day = Day 1). The animals were caged individually on wood shavings in a bio-clean room maintained at a constant temperature (20–24°C), relative humidity (40–50%), and photoperiod (12 hr light/day). The rats were given unlimited access to commercial lab chow (Purina) and tap water.

Parturition occurred on gestation Day 22 and the following day was designated as postnatal Day 1. To minimize genetic differences between litters, all pups were removed from their nests on Day 1, and segregated by sex. Five male and five female pups were then randomly selected from the total pool and placed with each dam. Litters were weaned on Day 22.

Pups were injected ip on Day 1, 8, 15, or 29 with 0, 30, or 48 mg/kg NaF in 0.85% saline (5 ml solution/kg body wt). Dose levels were chosen after conducting a pilot study using death as the measured endpoint. A dose causing a low frequency of deaths, and one causing no mortality were desired. The 48 mg/kg dose was lethal to 11 of 20 rats at Day 36, 6 of 20 at Day 29, and 2 of 20 at Day 22. The 30 mg/kg dose was the highest dose which did not cause any mortality in any age group. Each dose group consisted of 40–50 animals from 4 or 5 litters at each age.

Urine collection. Urine was obtained by stimulating the perineal region to induce micturition and collecting the expressed urine in a capillary pipet (Kavlock and Gray, 1982). This method allows quantitative collection of urine up to Day 13, as rats younger than this cannot voluntarily micturate. Urine flow was determined in Day 29 rats by placing them in metabolism cages for extended periods.

Renal functions in suckling rats. Indices of renal function were determined at 24, 48, and 120 hr postinjection (pi) in rats treated on Day 1, 8, or 15. The pups' bladders were voided and the osmolality of their urine was determined by vapor pressure osmometry (Wescor Model 5100 CXR, Wescor, Inc., Logan, Utah). The pups were then isolated from their dams, and hence were fluid deprived, for a 4-hr period. During this time, they were kept in beakers filled with wood shavings suspended in a 35°C water bath. At the end of the 4-hr hydropenia, urine was collected quantitatively, and urine osmolality was again measured. Urine chloride content was measured coulometrically (Radiometer Copenhagen CMT-10 chloride titrator, Copenhagen, Denmark) and urine pH was determined. Clinical reagent strips (Ames Co., Elkhart, Ind.) were used to qualitatively detect the presence of protein, glucose, and occult blood in the urine. No quantitation of these parameters was attempted for suckling rats.

Renal function in weanling rats. Weanling rats were placed in metabolism cages (Sybron Nalge, Rochester, N.Y.) on Day 28 and injected ip with NaF on Day 29, as previously described. The rats were maintained in the metabolism cages until 120 hr pi. The diet in the metabolism cages consisted of powdered lab chow mixed with corn oil (5/1, w/v). The semisolid consistency of this mixture prevented the powdered diet from falling into urine collection vessels and contaminating the samples. Water consumption, urine flow, and body weight were measured daily. Each 24-hr urine sample was assessed for osmolality, pH, and chloride content. Urine protein was measured colorimetrically by Coomassie blue (Bio-Rad Laboratories, Richmond, Calif.) binding (Bradford, 1976). This method allows quantitative measurement of urine protein concentration and was employed in this age group because protein is a normal urinary constituent in rats after weaning (Daston *et al.*, 1983), making clinical reagent strips unusable for this parameter. Clinical test strips (Ames Co., Elkhart, Ind.) were used to detect glucose and hemoglobin in the urine. In order to determine urine concentrating ability, the rats were water deprived for 24 hr, from 96 to 120 hr pi.

Litter mates of the animals placed in the metabolism cages were injected with NaF on Day 29 and sacrificed 24, 48, and 120 hr pi. Their kidneys were weighed and preserved for histological examination.

Histology. Suckling rats were sacrificed by decapitation following the 4-hr hydropenia and weanling Day 29 rats were sacrificed by cervical dislocation. Kidneys were removed, weighed, and fixed in a 5% formaldehyde, 5% acetic acid, 70% ethanol solution. The fixed kidneys were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Pathological examination was carried out independently (Experimental Pathology Laboratories, Raleigh, N.C.).

Statistics. Data were analyzed using the general linear models procedure available on the Statistical Analysis System (Helwig and Council, 1979). Differences between treatment groups were compared in each age group for each parameter using two-way analysis of variance. For any given parameter, if no significant interaction between observation time (hr pi) and dose of NaF was detected, then the data for all observation times were pooled within each dose group for statistical comparison. Otherwise, comparisons were made between dose groups at each observation time. Differences between dose groups were determined by

comparing least square means. Dose–response relationships were determined by analysis of variance. Prior to dosing, the mean body weight of the Day 29 rats of the 48 mg/kg group was significantly lower than the control mean. Therefore, predosing weight was used as a covariate to correct for this difference in subsequent body weight measurements. All data were reported as means ± standard errors of the means.

RESULTS

Exposure on Days 1, 8, or 15

Injection of 30 or 48 mg/kg NaF had little effect on rats less than three weeks of age. Functional and morphological data from Days 1, 8, and 15 rats are presented in Tables 1, 2, and 3, respectively.

Neither dose of NaF produced any mortality in these three age groups. Body weight and kidney weight were only affected in rats injected on Day 1. Both doses of NaF caused a significant decrease in body weight and kidney weight. Kidney/body weight ratio was increased by 6% in the 48 mg/kg group 120 hr pi on Day 1 (Table 1).

Basal urinary osmolality, measured immediately after isolating the pups from their dams, was unaffected by NaF exposure, but tended to be slightly higher in the treated rats. The volume of urine produced over a 4-hr period of hydropenia was increased by 50% over controls 120 hr pi of 48 mg/kg NaF on Day 8 (Table 2). Urine volume was not measured in rats treated on Day 15.

Exposure to NaF did not decrease the ability of the kidney to concentrate solutes in response to fluid deprivation. In fact, the urine osmolality was actually increased 120 hr pi in the 48 mg/kg group on Day 15 (Table 3), and in both dose groups 120 hr pi on Day 1 (Table 1).

Urine pH was significantly decreased by both doses of NaF in pups treated on Day 1 or 8 (Tables 1 and 2). Urinary chloride excretion is normally high on the first day after birth, then decreases until weaning. Chloride excretion was decreased to 68% of the control value in the 48 mg/kg group 24 hr pi on Day 1 (Table 1). No glucose, hemoglobin, or abnormal amounts of protein were detected in the urine of animals in these age groups.

There were no pathological changes observed in histological preparations from animals injected with NaF on Day 1, 8, or 15.

Exposure on Day 29

No mortality was observed in rats exposed to either dose of NaF on Day 29. This is in contrast to our preliminary dose-finding results where the 48 mg/kg dose killed 30% of the rats injected on Day 29. The body weights of the rats in the 48 mg/kg group were significantly lower than controls prior to dosing on Day 29; therefore, Day 29 body weight was used as a covariate in statistically analyzing subsequent changes in body weight. Compensating for predosing body weight in this manner, there was no treatment-related effect on weight (Table 4). Kidney weight was increased in the 48 mg/kg group, and kidney/body weight ratio was 10–15% higher than the controls in this dose group (Table 4).

TABLE 1

EFFECTS OF NaF INJECTION ON DAY 1 ON RENAL PARAMETERS

Dose (mg/kg)	Time Postinjection (hr)	n	Body wt[a] (g)	Kidney wt[a] (mg)	Kidney wt/ body wt (%)	Urine osmolality (mOsM)		Relative urine volume (μl/g body wt/hr)	Urine pH[a]	Urinary chloride excretion (nEq/g body wt/hr)
						Basal	After 4 hr hydropenia			
0	24	16	6.8 ± 0.2	77 ± 3	1.12 ± 0.03	266 ± 17	420 ± 16	2.79 ± 0.23	5.63 ± 0.16	154 ± 9
	48	17	8.1 ± 0.3	98 ± 4	1.22 ± 0.03	263 ± 13	496 ± 17	3.01 ± 0.28	5.85 ± 0.09	57 ± 13
	120	17	13.2 ± 0.3	179 ± 4	1.36 ± 0.02	408 ± 30	710 ± 20	1.95 ± 0.13	5.65 ± 0.07	21 ± 4
30	24	17	6.7 ± 0.1[b]	71 ± 3[b]	1.07 ± 0.04	266 ± 18	368 ± 14	2.67 ± 0.15	5.36 ± 0.07[b]	147 ± 14
	48	16	7.5 ± 0.2[b]	91 ± 4[b]	1.20 ± 0.04	276 ± 12	478 ± 14	3.04 ± 0.28	5.73 ± 0.12[b]	72 ± 9
	120	16	12.3 ± 0.3[b]	168 ± 5[b]	1.37 ± 0.02	447 ± 23	813 ± 43[b]	1.58 ± 0.13	5.50 ± 0.07[b]	29 ± 6
48	24	17	6.4 ± 0.2[b]	66 ± 2[b]	1.04 ± 0.02	296 ± 23	399 ± 21	2.32 ± 0.19	5.40 ± 0.07[b]	105 ± 12[b]
	48	17	7.8 ± 0.2[b]	93 ± 3[b]	1.19 ± 0.02	273 ± 28	530 ± 16	2.75 ± 0.21	5.87 ± 0.08[b]	78 ± 17
	120	16	12.0 ± 0.3[b]	173 ± 4[b]	1.44 ± 0.02[b]	389 ± 26	820 ± 37[b]	1.68 ± 0.15	5.43 ± 0.06[b]	28 ± 5

[a] There was no statistical interaction between dose of NaF and hours postinjection for this parameter; therefore, all observations within each dose were pooled.
[b] Significantly different from controls by general linear models procedure, $P < 0.05$

TABLE 2
EFFECTS OF NaF INJECTION ON DAY 8 ON RENAL PARAMETERS

Dose (mg/kg)	Time postinjection (hr)	n	Body wt[a] (g)	Kidney wt[a] (mg)	Kidney wt/ body wt[a] (%)	Urine osmolality (mOsM)		Relative urine volume (μl/g body wt/hr)	Urine pH[a]	Urinary chloride excretion[a] (nEq/g body wt/hr)
						Basal	After 4 hr hydropenia[a]			
0	24	16	17.1 ± 0.5	222 ± 6	1.30 ± 0.02	289 ± 19	631 ± 28	1.43 ± 0.11	6.50 ± 0.17	29 ± 5
	48	17	20.0 ± 0.5	266 ± 9	1.33 ± 0.02	235 ± 10	586 ± 39	2.18 ± 0.20	6.97 ± 0.22	23 ± 5
	120	17	27.9 ± 0.8	351 ± 15	1.25 ± 0.03	458 ± 52	889 ± 45	1.70 ± 0.18	5.79 ± 0.09	17 ± 4
30	24	17	16.8 ± 0.6	215 ± 9	1.28 ± 0.02	297 ± 23	665 ± 55	1.56 ± 0.17	6.23 ± 0.09[b]	29 ± 6
	48	16	19.3 ± 0.6	259 ± 9	1.34 ± 0.02	292 ± 33	757 ± 64	1.74 ± 0.15	6.59 ± 0.22[b]	26 ± 6
	120	17	28.5 ± 0.6	353 ± 11	1.24 ± 0.02	353 ± 11	887 ± 38	2.08 ± 0.19	5.76 ± 0.05[b]	20 ± 4
48	24	17	18.3 ± 0.4	232 ± 8	1.27 ± 0.03	333 ± 35	754 ± 56	1.38 ± 0.10	6.18 ± 0.11[b]	28 ± 5
	48	17	20.6 ± 0.6	271 ± 6	1.32 ± 0.02	265 ± 22	665 ± 30	2.20 ± 0.13	6.15 ± 0.14[b]	28 ± 9
	120	15	29.3 ± 0.7	372 ± 9	1.27 ± 0.02	511 ± 34	876 ± 43	2.56 ± 0.24[b]	5.71 ± 0.09[b]	23 ± 5

[a] There was no statistical interaction between dose of NaF and hours postinjection for this parameter; therefore, all observations within each dose were pooled.
[b] Significantly different from controls using general linear models procedure, $P < 0.05$.

The volume of urine produced over each 24-hr period was measured. Urine volume was significantly increased over control levels in the 30 mg/kg group 24 and 72 hr pi, and at all observation times in the 48 mg/kg dose group (Table 5). Urine volume in the 48 mg/kg group was at least twice the control value at 24 and 48 hr pi. The animals in the high-dose group produced 33% more urine than controls during the period of water deprivation 96–120 hr pi (Table 5). The NaF-induced diuresis was significantly dose related ($P < 0.05$) at all observation times from 24 to 96 hr pi. Along with the polyuria, water consumption was also increased in the 48 mg/kg group at all observation times after treatment (Table 5). The polydypsia was significantly dose related ($P < 0.001$).

Urine osmolality was decreased by the NaF treatment from 24 to 72 hr and at 120 hr pi in the 30 mg/kg group, and at all times after dosing with 48 mg/kg. Urine concentrating ability in the 30 mg/kg group was largely restored by 96 hr pi; however, these animals were still compromised in their ability to increase urinary osmolality in response to water deprivation from 96 to 120 hr pi (Table 5). The decrease in urine osmolality was significantly dose related ($P < 0.05$) at all observation times.

Urinary pH was increased in both dose groups after NaF treatment on Day 29 (Table 5). This is in contrast to the effect on Day 1 and 8 pups, where urinary pH decreased as a result of NaF exposure. The increase in urinary pH after injection on Day 29 was significantly dose related ($P < 0.03$). Urinary chloride excretion was decreased in a dose-related manner in both the 30 and 48 mg/kg dose groups 24 and 48 hr pi. Subsequently, chloride excretion was normal in these groups over the next 2 days, when the rats were normally hydrated. During 24 hr of water deprivation from 96 to 120 hr pi, the NaF-treated rats excreted 19% more chloride than did the controls, a significant increase (Table 5).

Glucosuria was observed in 5 of 13 rats in the 48 mg/kg group 24 hr pi. Hemoglobin was detected in the urine of 2 of 13 rats at 24 hr pi, and 1 of 13 at 48 hr pi in the 48 mg/kg group. Protein is a normal urinary constituent after weaning in the rat. Neither dose of NaF caused an increase in urinary protein concentration above the control level (Table 5).

Some renal tubular lesions were apparent upon histological examination in animals of both dose groups. The most frequently observed lesion was proximal tubular necrosis. This was first noticed 24 hr pi and persisted through 48 hr pi with eosinophilic debris present in the lumina of the damaged tubules. In the 30 mg/kg group, 60% of the rats sacrificed 24 hr pi and 67% of those sacrificed 48 hr pi had this histological damage. In the 48 mg/kg group, 96% of the rats had proximal tubular necrosis during the first 2 days after dosing. By 120 hr pi, extensive regeneration of the proximal tubular epithelium was observed in animals of both dose groups.

DISCUSSION

Exposure to NaF caused changes in histological and renal functional parameters in weanling rats. Although some treatment related effects were observed in suckling rats, these were not severe, and it appears that prior to weaning the rat kidney is relatively insensitive to the doses of Naf used in this study. Renal

TABLE 3
EFFECTS OF NaF INJECTION ON DAY 15 ON RENAL PARAMETERS

Dose (mg/kg)	Time postinjection (hr)	n	Body wt[a] (g)	Kidney wt[a] (mg)	Kidney wt/ body wt[a] (%)	Urine osmolality (mOSM)		Urine pH[a]
						Basal	After 4 hr. hydropenia	
0	24	13	30.1 ± 1.3	366 ± 18	1.2[illegible] ± 0.03	366 ± 18	978 ± 78	5.86 ± 0.06
	48	14	34.4 ± 1.3	436 ± 20	1.27 ± 0.02	352 ± 23	954 ± 60	6.20 ± 0.08
	120	11	46.1 ± 1.4	573 ± 17	1.24 ± 0.02	656 ± 101	1364 ± 72	5.79 ± 0.13
30	24	17	32.1 ± 0.7	392 ± 10	1.22 ± 0.02	414 ± 33	1046 ± 53	5.93 ± 0.06
	48	16	35.9 ± 0.6	443 ± 10	1.24 ± 0.02	389 ± 26	1097 ± 38	6.27 ± 0.11
	120	17	45.7 ± 1.0	560 ± 13	1.23 ± 0.02	532 ± 59	1541 ± 63	5.93 ± 0.12
48	24	17	32.4 ± 1.4	392 ± 20	1.21 ± 0.02	394 ± 30	910 ± 40	5.75 ± 0.06
	48	16	36.5 ± 1.0	449 ± 15	1.23 ± 0.02	374 ± 26	1067 ± 29	6.26 ± 0.09
	120	14	47.7 ± 1.1	599 ± 24	1.25 ± 0.03	512 ± 78	1742 ± 88[b]	5.78 ± 0.08

[a] There was no statistical interaction between dose of NaF and hours postinjection for this parameter; therefore, all observations within each dose were pooled.

[b] Significantly different from controls by general linear models procedure, $P < 0.05$.

TABLE 4
EFFECTS OF NaF INJECTION ON DAY 29 ON MORPHOLOGICAL PARAMETERS

Dose (mg/kg)	Time postinjection (hr)	n	Body wt[a,b] (g)	Kidney wt[a] (mg)	Kidney wt/ body wt[a] (%)
0	24	10	94.4 ± 3.2	1064 ± 60	1.12 ± 0.03
	48	11	99.3 ± 1.2	1042 ± 34	1.05 ± 0.02
	120	9	120.3 ± 1.6	1254 ± 36	1.04 ± 0.03
30	24	10	92.8 ± 2.6	1033 ± 33	1.11 ± 0.03
	48	9	100.6 ± 2.3	1069 ± 36	1.06 ± 0.03
	120	9	115.6 ± 3.1	1208 ± 41	1.05 ± 0.02
48	24	13	85.6 ± 2.3	1039 ± 34[c]	1.22 ± 0.03[c]
	48	12	96.0 ± 2.4	1160 ± 29[c]	1.21 ± 0.03[c]
	120	11	116.2 ± 4.3	1402 ± 61[c]	1.21 ± 0.04[c]

[a] There was no significant interaction between dose of NaF and hours postinjection for this parameter; therefore, all observations within each dose were pooled.
[b] Body weights were analyzed using body weight at the time of injection as a covariate.
[c] Significantly different from controls by general linear models procedure, $P < 0.05$.

parameters were affected by NaF in Day 29 rats, the oldest animals in this study. In this age group, the ability to produce a concentrated urine was impaired; urine volume and water consumption were increased; chloride excretion was altered; kidney–body weight ratio was increased; and histological lesions of the proximal tubule were observed.

Diuresis and polydypsia in response to NaF administration have been previously reported in adult rats (Whitford and Taves, 1971; Mazze, 1976). This is associated with a diminished solute gradient in medullary tissue (Frascino, 1972; Whitford and Taves, 1973; Rush and Willis, 1982). However, the renal site of action of F^- has not been established. There is some evidence from the present study that the proximal tubule is the site of toxicity, since histologically observable lesions are present in the proximal tubular epithelium. This has been noted previously in mature rats (Kosek *et al.*, 1972; Mazze *et al.*, 1973). We also detected glucose in the urine of several individuals receiving 48 mg/kg NaF on Day 29, again suggesting impairment of solute reabsorption by the proximal tubule. This would result in an osmotic diuresis. It should be stated that NaF causes hyperglycemia in rats (Hodge and Smith, 1965). This may result in the glucosuria and diuresis observed here even without proximal tubular dysfunction.

Inhibition of medullary transport functions would also account for the observed polyuria. An increased fractional excretion of sodium, indicating an inhibition of NaCl transport in the ascending limb of the loop of Henle, has been reported (Roman *et al.*, 1977; Rush and Willis, 1982). On the other hand, others have reported that the functional excretion of sodium is unchanged (Whitford and Taves, 1971) or even decreased (Mazze *et al.*, 1972) by NaF treatment. We observed a decrease in urinary chloride excretion in Day 29 rats 24 and 48 hr after NaF injection. This appears to support the view that chloride reabsorption in the ascending limb is not inhibited by F^-. However, our total chloride excretion data

TABLE 5
EFFECTS OF NaF INJECTION ON DAY 29 ON RENAL PARAMETERS

Dose (mg/kg)	Time postinjection (hr)	n	Body wt[a,b] (g)	Water consumption[a] (ml/100 g body wt/day)	Urine volume (ml/100 g body wt/day)	Urine osmolality (mOsM)	Urine pH[a]	Urinary chloride excretion (mEq/100 g body wt/day)	Urinary protein[a]
0	0	12	87.0 ± 2.7	18.6 ± 1.1	7.38 ± 0.41	1574 ± 61	7.23 ± 0.08	1.96 ± 0.07	11.40 ± 2.88
	24		93.2 ± 2.8	25.2 ± 4.9	6.96 ± 0.30	1788 ± 175	6.52 ± 0.36	2.29 ± 0.06	9.05 ± 2.07
	48		101.2 ± 3.0	18.0 ± 0.8	5.62 ± 0.35	1947 ± 115	6.72 ± 0.07	1.81 ± 0.04	13.25 ± 1.67
	72		107.3 ± 2.9	18.8 ± 0.8	6.24 ± 0.41	1969 ± 130	6.85 ± 0.19	2.10 ± 0.07	10.15 ± 0.72
	96		112.1 ± 3.5	17.4 ± 0.9	5.93 ± 0.35	1949 ± 115	6.85 ± 0.21	1.96 ± 0.08	8.03 ± 1.76
	120		103.3 ± 3.0	[c]	3.14 ± 0.44	2788 ± 203	6.63 ± 0.20	1.30 ± 0.10	ND[e]
30	0	11	90.2 ± 2.7	17.9 ± 1.2	6.83 ± 0.59	1518 ± 88	7.22 ± 0.10	1.81 ± 0.08	6.00 ± 1.78
	24		94.7 ± 3.2	23.4 ± 0.8	12.14 ± 0.65[d]	959 ± 57[d]	7.13 ± 0.09[d]	1.76 ± 0.05[d]	7.82 ± 1.99
	48		102.2 ± 3.3	20.2 ± 0.9	7.45 ± 0.65	1331 ± 103[d]	6.65 ± 0.07[d]	1.59 ± 0.03[d]	6.82 ± 1.51
	72		107.9 ± 3.3	20.4 ± 0.7	8.47 ± 0.65[d]	1473 ± 115[d]	7.41 ± 0.30[d]	2.01 ± 0.07	7.55 ± 0.83
	96		114.9 ± 3.8	18.4 ± 0.5	6.96 ± 0.45	1654 ± 108	7.26 ± 0.27[d]	1.92 ± 0.05	10.12 ± 3.24
	120		105.6 ± 3.7	[c]	3.98 ± 0.29	2264 ± 132[d]	6.68 ± 0.18[d]	1.54 ± 0.06[d]	ND
48	0	13	80.5 ± 1.5	18.7 ± 1.6	6.73 ± 0.70	1616 ± 88	7.44 ± 0.11	1.91 ± 0.07	11.69 ± 4.34
	24		83.4 ± 1.5	29.3 ± 1.2[d]	16.29 ± 0.97[d]	725 ± 50[d]	7.15 ± 0.10[d]	1.63 ± 0.10[d]	16.96 ± 6.92
	48		90.3 ± 1.9	26.6 ± 1.6[d]	11.17 ± 1.07[d]	899 ± 74[d]	6.79 ± 0.21[d]	1.50 ± 0.06[d]	14.48 ± 6.08
	72		96.9 ± 1.9	25.6 ± 1.8[d]	9.76 ± 0.79[d]	1266 ± 95[d]	7.55 ± 0.30[d]	2.00 ± 0.09	10.06 ± 1.36
	96		102.9 ± 2.2	21.3 ± 0.9[d]	7.77 ± 0.57[d]	1515 ± 101[d]	7.11 ± 0.23[d]	1.97 ± 0.07	6.08 ± 1.18
	120		92.7 ± 2.1	[c]	4.18 ± 0.29[d]	2215 ± 138[d]	6.68 ± 0.16[d]	1.53 ± 0.06[d]	ND

[a] There was no significant interaction between dose of NaF and hours postinjection for this parameter; therefore, all observations within each dose were pooled.
[b] Body weights were analyzed using body weight at 0 hr postinjection as a covariate.
[c] Animals were water deprived for the final 24 hr of the experiment.
[d] Significantly different from controls by general linear models procedure, $P < 0.05$.
[e] Not determined

may not be comparable to fractional excretion data, since glomerular filtration rate (GFR) may have been affected by the NaF exposure. GFR has been reported to decrease (Whitford and Taves, 1973), increase (Roman *et al.*, 1977), or remain constant (Frascino, 1972; Rush and Willis, 1982) after NaF treatment. It is of interest that in the present study, chloride excretion returned to control levels 72 hr after NaF treatment in normally hydrated rats. This corresponds to the time when the proximal tubule epithelium was regenerating, and hemoglobin and glucose were no longer present in the urine. However, fluid deprivation from 96 to 120 hr pi caused an increased excretion of chloride in the treated rats. This suggests that chloride reabsorption may be affected by NaF exposure, but this effect may require a few days to develop.

Diuresis induced by NaF treatment is unresponsive to vasopressin (Mazze *et al.*, 1972), and a decreased responsiveness of medullary tissue *in vitro* to vasopressin has also been noted after NaF (Rush and Willis, 1982), suggesting that NaF impaired water movement out of the collecting tubule. In the present study, we noted that even though the diuresis and urine-concentrating deficit were subsiding by 96 hr pi in both dose groups treated on Day 29, when deprived of fluid from 96 to 120 hr pi, these animals failed to respond to the same degree as controls. Their urine osmolality was low, and urine volume was increased during this period. Since vasopressin stimulates renal conservation of water during hydropenia, it is possible that the rats given NaF in this study have a decreased responsiveness to this hormone.

The immature rats in this study were largely unresponsive to NaF nephrotoxicity. Toxic effects were minimal in the three age groups under 3 weeks old, and virtually none of the renal effects noted in older animals were observed in the pups. There may have been an age-related increase in urine volume in response to NaF. Urine volume was increased on Day 13 in rats injected with 48 mg/kg on Day 8, and profound diuresis was observed after NaF treatment on Day 29. We were unable to quantitatively collect urine from rats injected on Day 15; therefore, it cannot be ascertained whether the increased urine volume in the Day 13 rats was an isolated event, or marked the chronological onset of the renal diuretic response to NaF during postnatal maturation. An age-related increase in mortality after NaF exposure has been previously reported in postweaning rats. Maynard *et al.* (1951) observed a steadily increasing mortality rate after an ip injection of 26 mg/kg NaF as rats aged from 1 month (0% mortality) to 1 year (92% mortality). deLopez and co-workers (1976) found that the acute oral LD_{50} of NaF decreased from 54 mg/kg in 80-g rats to 31 mg/kg in 250-g rats. It has also been reported that accidental ingestion of NaF by human children in Austria is a common occurrence, but that these poisonings do not usually present a serious risk (Eichler *et al.*, 1982).

One of the few treatment-related effects noted in the young animals was a decrease in urine pH observed after injection of NaF on Day 1 or 8. We have previously observed a dose-related decrease in urinary pH at these ages after treatment with another nephrotoxin, $HgCl_2$ (Daston *et al.*, 1983, 1984), and this may be a generalized response of the immature rat kidney to toxic insult. Renal clearance of F^- is highy dependent on pH. Below a pH of 5.6, most fluoride is

nonionized, probably present as a HF, and only 5% of filtered fluoride is excreted at this pH. Above pH 5.6, the percentage of ionized fluoride increases, and there is an increase in fractional excretion (Whitford *et al.*, 1976; Whitford and Pashley, 1979). The urinary pH of suckling rats is markedly more acidic than that of weanlings, and is often less than pH 6. Furthermore, urine pH is decreased in these animals after NaF treatment. Thus, the degree of ionization and therefore the renal handling of fluoride would be markedly different in suckling and weanling rats. However, the decreased urinary pH would favor reabsorption of fluoride and it is not clear how this would protect the immature rat kidney from toxicity, unless more rapid removal of fluoride from the filtered fluid protects the luminal surfaces of the tubular epithelium further downstream.

An alternative reason for the lack of sensitivity of the immature rats is that the distribution patterns of F^- change with age. F^- has a marked affinity for hard tissues and is rapidly cleared from the blood and sequestered in bone and teeth (Armstrong *et al.*, 1970; Hall *et al.*, 1977). It has been reported that the weanling rat (21–30 days) has a great capacity to concentrate F into its hard tissues, that this capability is apparently associated with rapid growth, and that there is a decrease in this capability with increasing age (Zipkin and McClure, 1952; Miller and Phillips, 1956). The plasma F^- concentration after a given dose of NaF also increases with age (deLopez *et al.*, 1976). Although these studies did not extend into the preweaning period, it is probable that the findings are relevant to the younger ages. Suckling rats undergo a rapid growth, approximately doubling their body weight each week for the first 3 weeks of life. Rapid growth and the formation of large quantities of bone have been associated with increased sequestering of F in weanlings (Zipkin and McClure, 1952), and the preweaning rats grow at an even faster rate. Thus, rapid removal of F^- by the growing skeletal system may explain the insensitivity of the young rat to NaF toxicity.

Another possible explanation of the insensitivity of the immature rat to NaF may be an intrinsic unresponsiveness of the immature rat kidney to toxic insult. The rat kidney is morphologically and functionally primitive at birth, and many physiological parameters mature during the early postnatal period. Differentiation of nephrons is incomplete until Day 11, and histological maturation continues into the third week of life (Baxter and Yoffey, 1948). Controlling for increases in kidney weight, GFR increases more than sevenfold in the rat during the first 3 weeks after birth (Horster and Lewy, 1970). Thus, the nephrons may be exposed to steadily increasing amounts of nephrotoxin as GFR increases with renal maturity. We have previously reported that the immature rat kidney is also less sensitive to $HgCl_2$, a proximal tubular toxin in adults, although as with NaF this insensitivity may also be attributable to differences in distribution to the kidney (Daston *et al.*, 1983, 1984).

The present study has investigated the toxicity of NaF to the postnatally developing kidney. Severe renal toxicity was observed only in Day 29 rats, the oldest animals used in this study. Renal dysfunction included diuresis, an inability to concentration urine in response to water deprivation, and altered chloride excretion. Glucose and hemoglobin were present in the urine. Kidney weight was

increased and histological lesions were observed in the proximal tubules. Possible explanations for the resistance of immature rats to NaF were discussed.

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